PB84-224450

Estimating the Acute Toxicity of Narcotic Industrial Chemicals to Fathead Minnows

(U.S.) Environmental Research Lab.-Duluth, MN

Jul 84

U.S. Department of Commerce
National Technical Information Service
NTIS

PB84-224450

EPA-600/D-84-190
July 1984

ESTIMATING THE ACUTE TOXICITY OF NARCOTIC INDUSTRIAL CHEMICALS TO FATHEAD MINNOWS

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TECHNICAL REPORT DATA *(Please read Instructions on the reverse before completing)*		
1. REPORT NO. EPA-600/D-84-190	2.	3. RECIPIENT'S ACCESSION NO. PB84 224450
4. TITLE AND SUBTITLE Estimating the Acute Toxicity of Narcotic Industrial Chemicals to Fathead Minnows		5. REPORT DATE 1984 6. PERFORMING ORGANIZATION CODE
7. AUTHOR(S) G.D. Veith, D.J. Call, and L.T. Brooke		8. PERFORMING ORGANIZATION REPORT NO.
9. PERFORMING ORGANIZATION NAME AND ADDRESS U.S. Environmental Protection Agency Environmental Research Laboratory-Duluth 6201 Congdon Boulevard Duluth, MN 55804		10. PROGRAM ELEMENT NO. 11. CONTRACT/GRANT NO.
12. SPONSORING AGENCY NAME AND ADDRESS Environmental Research Laboratory Office of Research and Development U.S. Environmental Protection Agency Duluth, MN 55804		13. TYPE OF REPORT AND PERIOD COVERED 14. SPONSORING AGENCY CODE EPA-600/03
15. SUPPLEMENTARY NOTES		

16. ABSTRACT

The overwhelming number of industrial chemicals either in commerce or in development precludes actual toxicity testing of each chemical and creates a need for initial screening techniques to focus efforts on those chemicals with the greatest potential for adverse environmental effects. One promising screening technique is the use of the structure-activity relationship to estimate aquatic toxicity from chemical structure.

Using five major classes of industrial chemicals from the Toxic Substances Control Act (TSCA) inventory, this paper presents a structure-activity relationship between the 96-h LC50 with fathead minnows and the n-octanol/water partition coefficient which can be computed from structure. Common industrial alcohols, ketones, alkyl halides, ethers, and benzenes are shown to cause lethality by the same mode of action of narcosis. Consequently, a single bilinear equation can predict adequately the 96-h LC50 of all classes of industrial chemicals to fathead minnows.

17. KEY WORDS AND DOCUMENT ANALYSIS		
a. DESCRIPTORS	b. IDENTIFIERS/OPEN ENDED TERMS	c. COSATI Field/Group

18. DISTRIBUTION STATEMENT	19. SECURITY CLASS *(This Report)*	21. NO. OF PAGES
Release to public	unclassified	10
	20. SECURITY CLASS *(This page)*	22. PRICE
	unclassified	

EPA Form 2220-1 (Rev. 4-77) PREVIOUS EDITION IS OBSOLETE

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The inventory of industrial chemicals includes a large proportion of relatively innocuous chemicals. Cost-effective hazard assessment methods must use rapid screening techniques to estimate toxicity and to focus testing resources on the more toxic chemicals. Although few papers concerned with the lethality of industrial chemicals to aquatic organisms discuss the modes of action, we have observed that narcosis is a predominant mechanism of lethality among the less toxic chemicals.

Narcosis is a reversible state of arrested activity of various protoplasmic structures under the influence of chemicals, and comprehensive reviews have been published [*1–3*].[3] Ferguson [*4*] proposed that when such a physiological effect is reversible, an equilibrium exists between the organism and the external phase, and the physiological effect is a function of external concentration; the toxic effect is physical in character. Physical toxicity will be exhibited by organic compounds at sufficient concentration unless it is masked by irreversible chemical effects related to specific functional groups within the molecule.

The physical nature of narcosis leads to effects which are chemically nonspecific, as evidenced by the narcotic action of gases [*5*], aliphatic and aromatic hydrocarbons, chlorinated hydrocarbons, alcohols, ethers, ketones, aldehydes, weak acids and bases, and some aliphatic nitro compounds [*1,3,6*].

Ferguson [*4*] and Mullins [*7*] proposed that the chemical thermodynamic activities of narcotics in blood at the point of equal effects are approximately equal, with some of the variability accounted for by variations in molecular volume. Because the chemical activity in blood is related to the chemical concentration in blood through the activity coefficient, which is difficult to measure, toxic responses are seldom based on chemical activity. The chemical activity of volatile narcotics in mammalian studies has been estimated from the partial pressure of the chemical causing narcosis through the expression

$$A_{nar} \cong P_{nar}/P^{\circ}$$

where A_{nar} is the thermodynamic activity for narcosis, P_{nar} is the partial pressure for narcosis, and P° is the partial pressure for the pure narcotic.

For nonvolatile chemicals in mammalian tests, A_{nar} is not easily determined. However, A_{nar} for nonvolatile chemicals in aquatic tests can be estimated from the expression

$$A_{nar} \cong \frac{C_{nar}}{C_s}$$

where C_{nar} is the narcotic concentration in water and C_s is the water solubility of the chemical. The estimation is expected to be valid provided the thermodynamic activity of the chemical in water is equal to the activity in the blood of the test organisms, that is, the chemical in water is at equilibrium with that in blood. Under these conditions, the acute toxicity of narcotics to fish can be expected to be directly related to the water solubility of the chemical. This work presents an evaluation of this model in estimating aquatic toxicity of industrial narcotics.

[3]The italic numbers in brackets refer to the list of references appended to this paper.

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Experimental Procedures

Chemicals for this study were selected from the Toxic Substances Control Act (TSCA) industrial inventory. The 96-h LC_{50} of the chemicals were determined for fathead minnows (*Pimephales promelas*) from the Environmental Research Laboratory-Duluth culture using flow-through proportional diluters [*8*] and test methods described by the U.S. Environmental Protection Agency Committee on Methods for Toxicity Tests with Aquatic Organisms (1975). Lake Superior water maintained at 25 ± 1°C was used in all tests. Routine measures of hardness and alkalinity of the test water yielded mean values of 56.3 and 42.2 mg/L calcium carbonate ($CaCO_3$), respectively. The arithmetic mean of the pH was 7.5, and dissolved oxygen was always greater than 60% of saturation.

Twenty-five 30-day-old fish, each weighing approximately 0.12 g, were randomly divided among twelve test tanks (a control and five different concentrations, each in duplicate). The fish were not fed during the test, and deaths were recorded after 1, 3, 6, 12, 24, 48, 72, and 96 h. The median lethal concentration, or LC_{50}, was computed using the trimmed Spearman-Karber method [*9*] on a PDP-11/70 computer.

Concentrations of chemicals in water were measured in each tank throughout the test. The water was analyzed either by ultraviolet spectroscopy or by direct aqueous injection on a gas chromatograph (GC). GC analyses were performed on a Hewlett-Packard 5730A gas chromatograph equipped with flame ionization detectors and 122-cm by 2-mm inside-diameter columns filled with the porous polymer Tenax GC (Applied Science, Riviera Beach, FL). The column temperature was varied for each chemical between 100 and 225°C to provide optimum analytical conditions.

Water solubility of the test chemical was obtained from the literature or determined experimentally. The chemicals were added to water in 500-mL flasks and gently shaken in a 25 ± 1°C water bath until the concentration was constant. Samples of saturated water were centrifuged for 30 min at 10 000 *g*, 25 000 *g*, and 40 000 *g* in separate centrifuge tubes. Water from each tube was removed for GC analysis. In general, the samples centrifuged at more than 10 000 *g* contained statistically identical concentrations to those centrifuged at 10 000 *g*, and therefore all water solubility values were treated as replicates.

The values for *n*-octanol/water partition coefficients were taken from the monograph of Hansch and Leo [*10*]. Those partition coefficients not previously measured were estimated by reverse-phase liquid chromatography, as described by Veith et al [*11*] or calculated using the fragment constant method of Hansch and Leo [*10*].

Results and Discussion

A series of alkyl alcohols belonging to a class previously demonstrated to exhibit narcosis were tested to determine relationships among aquatic toxicity, water solubility, and log *P* of the chemicals. The results in Table 1 show that the log LC_{50} (in moles per litre) ranged from −0.06 for methanol to −5.27 for 1-dodecanol. 1-Tridecanol produced no mortality at its solubility limit of 0.33 mg/L. The data show that the toxicity of the alcohols (expressed as log LC_{50} in moles per litre) increases with increasing log *P* and decreasing water solubility (log *S* in moles per litre). The relationship is linear for homologues below 1-decanol and becomes nonlinear above decanol. The data are presented

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TABLE 1—*Water solubility, partition coefficient, and acute toxicity of common alcohols to fathead minnows.*

Chemical	96-h LC_{50}, mg/L	Log LC_{50}[a]	C_s, mg/L	Log C_s[a]	Log P	LC_{50}/C_s	A_{nar}[b]
Methanol	28 200	−0.06	[d]	[d]	−0.66	...	0.030
Ethanol	14 700	−0.51	[d]	[d]	−0.16	...	0.027
2-Propanol	10 000 (9 640)[c]	−0.80	[d]	[d]	0.14	...	0.025
1-Butanol	1 740	−1.63	74 700	0.03	0.88	0.023	0.022
1-Hexanol	97.2	−3.02	6 270	−1.21	2.03	0.015	
1-Octanol	13.4	−4.00	587	−2.35	3.03	0.023	0.020
1-Nonanol	5.7	−4.40	158	−3.00	3.53	0.036	
1-Decanol	2.3	−4.84	34	−3.67	4.03	0.068	0.055
1-Undecanol	1.04	−5.22	8.5	−4.31	4.53	0.122	
1-Dodecanol	1.01	−5.27	1.9	−4.99	5.00	0.532	
1-Tridecanol	[e]	[e]	0.33	−5.78	5.51	>1.0	

[a]Expressed as moles per litre.
[b]Calculated from $A_{nar} = LC_{50}$ (m/L) × activity coefficient, presented in Amidon et al [13].
[c]Duplicate toxicity tests in parenthesis.
[d]Miscible with water.
[e]No fish mortality in saturated solution.

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graphically in Fig. 1, for which the coordinates selected encompass essentially all of the common industrial chemicals. The square data points (Fig. 1) denote the water solubility for alcohols which are not miscible with water. The linear relationship with log *P*, presented by Hansch et al [*12*], was extended to include some of the more insoluble alcohols such as dodecanol and tridecanol. The graph in Fig. 1 identifies an aquatic toxicity space as only that region below the water solubility line. In the region above the solubility line, the acute toxicity would be greater than the solubility and would not be measurable under equilibrium conditions.

The acute toxicity and water solubility are covariant with respect to log *P* values less than 4.0 and have slopes of −1.04 and −1.09, respectively. The fact that toxicity is a relatively constant fraction of water solubility (chemical activity) suggests that the "Ferguson principle" of similar chemical activities producing similar effects in mammals is equally true for fish. Since narcosis in mammals is generally observed at chemical activities in the blood of 0.01 to 0.04, narcosis in fish would be expected at 0.01 to 0.04 of the water solubility if the fish were similar in sensitivity and an equivalent equilibrium existed be-

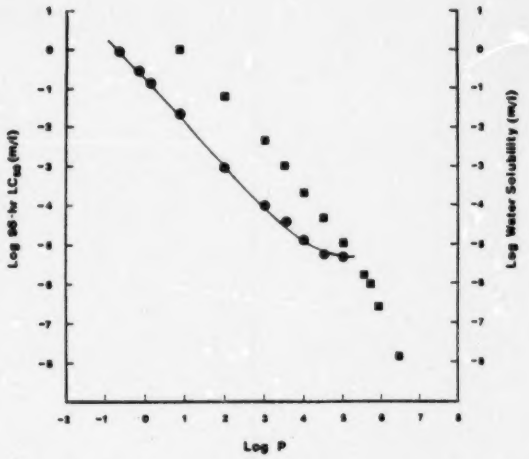

FIG. 1—*Bilinear relationship between water solubility* (squares) *and 96-h* LC_{50}*s* (circles) *with log* P *for aliphatic alcohols.*

tween the water and the fish blood. Table 1 shows that the ratio of LC_{50}/C_s varies from 0.015 for hexanol to 0.036 for nonanol. The activity (LC_{50}/C_s) of higher homologues increases until the LC_{50} is greater than water solubility. The activity of miscible chemicals cannot be estimated from solubility; however, chemical activity at the LC_{50} can also be estimated by multiplying the LC_{50} by the activity coefficient of the chemicals. Although activity coefficient data in the literature are scarce, Amidon et al [*13*] presented coefficients for six of these alcohols. The activities necessary for narcosis in fish computed from the activity coefficients are presented in the final column of Table 1. Not only do these estimates show excellent agreement with the estimates from water solubility, but also the mean activity of all lower homologues is approximately 0.025.

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TABLE 2—*Acute toxicity of other industrial chemicals to fathead minnows.*

Chemical	96-h LC_{50}, mg/L	Log LC_{50}	Log *P*
ALCOHOLS			
2-Methyl-1-propanol	1 460	1.29	0.68
1-Chloro-2-propanol	244	−2.58	0.85
2,2,2-Trichloroethanol	298	−2.70	1.39
2,3-Dibromopropanol	71	−3.49	1.52
Cyclohexanol	705	−2.15	1.23
2-Methyl-2,4-pentanediol	10 700	−1.03	−0.23
2-Phenoxyethanol	346	−2.06	1.16
KETONES			
Acetone	8 140 (7 310)[a]	−0.85	−0.24
2-Butanone	3 200	−1.35	0.285
2-Pentanone	1 530	−1.75	0.790
2-Octanone	37	−3.54	2.77
5-Nonanone	31	−3.57	2.79
2-Decanone	5.7 (4.2)[a]	−4.44	4.24
7-Tridecanone	[b]	[b]	5.53
3-Methyl-2-butanone	868	−2.00	0.590
5-Methyl-2-hexanone	158	−2.86	1.89
4-Methyl-2-pentanone	509	−2.29	1.25
6-Methyl-5-heptene-2-one	85.8	−3.17	2.18
3,3-Dimethyl-2-butanone	86	−3.07	1.82
Cyclohexanone	630 (732)[a]	−2.19	0.810
2-Butanone oxime	844	−2.01	0.89
Acetophenone	161	−2.87	1.73
Benzophenone	14.3 (15.2)[b]	−4.09	3.18
2,3,4-Trichloroacetophenone	2.0	−5.00	3.66
2,4-Dichloroacetophenone	11.7	−4.16	2.95
ETHERS			
Triethylene glycol	69 800	−0.33	−1.24
2-(2-Ethoxy ethoxy) ethanol	26 400	−0.71	−0.08
tert-Butylmethyl ether	706	−2.10	1.06
Diisopropyl ether	91.7	−3.05	1.74
Di-*n*-butyl ether	32.5	−3.60	3.05
2,6-Dimethoxytoluene	20.5	−3.88	2.87
Diphenyl ether	4.0	−4.63	4.21
Dipentyl ether	3.2	−4.70	4.17
p-Nitrophenyl phenyl ether	2.7	−4.91	3.93
Dioctyl ether	[b]	[b]	
ALKYL HALIDES			
1,2-Dichloroethane	118	−2.92	1.76
1,1,2-Trichloroethane	81.7	−3.21	2.28
1,1,2-Trichloroethylene	44.1	−3.48	2.42
1,1,2,2-Tetrachloroethane	20.3	−3.92	2.66
Tetrachloroethylene	13.5	−4.08	2.95
Pentachloroethane	7.3	−4.44	3.58
Hexachloroethane	1.5	−5.19	4.23
BENZENES			
1,3-Dichlorobenzene	7.8	−4.28	3.38
1,4-Dichlorobenzene	4.0	−4.56	3.35
1,2,4-Trichlorobenzene	2.9	−4.80	4.20
1,2,3,4-Tetrachlorobenzene	1.1	−5.30	4.94
Pentachlorobenzene	[b]	[b]	5.69
Hexachlorobenzene	[b]	[b]	6.44

[a]Duplicate toxicity test results in parenthesis.
[b]No fish mortality in saturated solution.

The increase in chemical activity observed for the higher homologues in this series may result from insufficient time to achieve a thermodynamic equilibrium between the water and lipid phases at water concentrations below 10^{-5} molar or from a decrease in the chemical activity of bulkier chemicals occupying larger molecular volumes [7]. Experiments to test these hypotheses are in progress in this laboratory.

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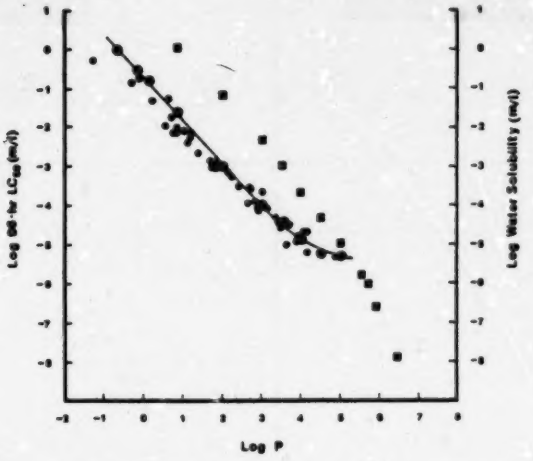

FIG. 2—*Relationship between the 96-h LC_{50}s* (small circles) *of narcotic chemicals and log* P *superimposed on a bilinear alcohol model.*

The data in Table 1 are summarized by the bilinear equation

$$\text{Log } LC_{50} = -1.09 \log P + 1.09 \log (0.000068\,P + 1) - 0.79$$

where $R^2 = 0.9986$. To test whether this relationship accurately models the toxicity of other industrial chemicals that are acutely toxic through narcosis, the 96-h LC_{50} of 45 other common chemicals from the industrial inventory were compiled in Table 2 and plotted on the alcohol model in Fig. 2. These data indicate general agreement between the narcosis equation using only log P as an independent variable and the observed LC_{50}.

Although these data indicate that chemicals exerting a common mode of action may be modeled jointly, it is likely that more accurate estimations will require an individual structure-activity relationship for each class of chemicals as well as the use of electronic and steric factors in addition to log P as molecular descriptors. The literature suggests that narcosis may not be a truly nonspecific mode of action but rather may consist of a series of different mechanisms such as membrane swelling and membrane depolarization, which produce the same apparent effects. Data generated to date on ester and phenol toxicity indicate that the mode of action appears to be narcosis; however, these groups are slightly more toxic than other narcotics. Consequently, the correlation with log P presented in Fig. 2 will likely become a "family" of lines for each class of chemical.

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